

Scientific Abstract

The aim of this clinical trial is to transfer by retroviral vector the cDNA for the human multidrug resistance gene, MDR-I, to hematopoietic progenitors from patients with relapsed or refractory high risk non-Hodgkin's lymphoma. The recipients of CD34+ cells transduced with retroviral vectors containing the cDNA for MDR-I in the previous human gene therapy clinical trials had all undergone high dose chemotherapy prior to the administration of transduced cells. Although gene marking was observed, it was relatively short-lived and at a very low level, and it was difficult to demonstrate chemotherapy protection. Our aim is to transfer the cDNA for MDR-1 to recipients who would receive minimal myeloablation to promote engraftment of transduced cells, and then be treated with dose escalating chemotherapy, including doxorubicin, vincristine, and etoposide. After the dose escalating chemotherapy, the patients would then undergo autologous stem cell transplant after high dose cyclophosphamide and total body irradiation. We will be using a new retroviral vector containing the cDNA for MDR-I, SF1m, that has not been used in the previous clinical trials, and has been demonstrated to result in high level expression in hematopoietic progenitors, superior to MMLV based vectors (1,2). The retroviral vector SF1m consists of the LTR of the spleen focus forming virus and the leader sequence of the murine embryonic stem cell virus, as well as the coding sequence of MDR-1 without remnants of untranslated regions. We have performed preclinical in vitro studies with human CD34+ cells and murine lineage depleted bone marrow cells, and in vivo studies in mice with SF1m retroviral supernatant derived from producer cells from a master cell bank, or using clinical grade supernatant. Human CD34+ cells isolated by the Miltenyi MACS system were transduced by co-culture and centrifugation. Seven percent of the cells transduced by centrifugation and 2.7% of the cells transduced by co-culture exhibited functional p-glycoprotein by rhodamine efflux assay (over control efflux). For SF1m transduced CD34+ cells, there was an initial transduction efficiency of 10% by colony PCR, but in the presence of doxorubicin, 57% of the colonies exhibited the transgene, and in the presence of etoposide, 17% of the colonies were positive. With transduction of bone marrow cells from male BALBc mice depleted of cells bearing lineage markers, the initial transduction efficiency was 5.6% by PCR analysis of CFU-GM. These cells were engrafted into female syngeneic recipients pre-treated with low dose (100cGy) total body irradiation. On day 18 after transplant, half the mice were treated with etoposide 20 mg/kg by intraperitoneal injection daily for 4 days, and half remained untreated. For the GFP control group, we obtained $27.0\pm2.8\%$ engraftment, for the GFP VP-16 group, $29.7\pm13.6\%$, for the MDR group $18.7\pm6.9\%$, and for the MDR + VP-16 group, $21.5\pm8.4\%$, analyzed by FISH for Y chromosome. One week after treatment, the average white blood count of the recipients of MDR-I transduced marrow was $6.4\pm1.2 \times 10^3/\text{mm}^3$ compared to $4.1\pm1.4 \times 10^3/\text{mm}^3$ in the control recipients of GFP transduced marrow. At 6 weeks post transplant, by rhodamine efflux assay, the bone marrow from the MDR-I transduced mice that did not receive etoposide exhibited 0.4% rhodamine efflux as compared to 6.5% by the group treated with etoposide, implying that there was enhanced survival of the cells containing the MDR-1 transgene under the selective pressure of the chemotherapy. Later experiments consisting of prestimulation with a cytokine combination, IL-3, SCF, TPO, Flt3, then transduction on Retronectin, followed by centrifugation led to even higher transduction efficiency, 88% of murine bone marrow

from 5-fluorouracil treated donors exhibited rhodamine efflux over control non-transduced, and 14.7% of human CD34+ cells effluxed rhodamine over the control. Thus, transduction of both human and murine cells with SF1m affords chemotherapy protection, and the transduced lineage depleted murine bone marrow cells can be successfully engrafted in minimally myeloablated murine recipients. We hope to achieve similar transduction efficiency in the clinical trial and achieve engraftment of transduced cells in minimally myeloablated autologous recipients. If this goal is achieved, we may be able to observe expansion of the MDR-1 expressing cells under the selective pressure of combination chemotherapy. As a safety measure, after the cycles of dose escalation chemotherapy, the patients will undergo myeloablative chemoradiotherapy and stem cell transplant with unmodified cells. It is believed that the high dose chemoradiotherapy will eliminate all transduced cells and thus remove the possibility of complications related to the theoretical risk of insertional mutagenesis or other effects related to expression of MDR-1 in hematopoietic cells.